CLAIMS

1. An *in vitro* method of determining activation or inactivation of the atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) hormonal systems, the method comprising simultaneously detecting the presence or amount of atrial and brain natriuretic peptide prohormones (proANP and proBNP) or fragments thereof in a sample.

2. A method according to claim 1, which comprises contacting the sample with a bi- or oligo- specific first binding substance that is able to bind to both:

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.

3. A method according to claim 1 which comprises contacting the sample with

- an agent comprising:

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iv) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

- a first binding substance which is able to bind to:

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iv) a fragment of (i) or (ii) which is at least 6 amino acids in length;

and

(c) the agent.

4. A method according to claim 3 wherein the first binding substance comprises:

(a) a bi- or oligo-specific binding substance; or

(b) a mixture of mono-specific binding substances.

5. A method according to any one of claims 2 to 4 wherein the first binding substance comprises:

(a) natriuretic receptor GC-A (SEQ ID NO: 33);

(b) homologous sequence having at least 70% identity to (a); or

(c) a fragment of (a) or (b) which is at least 400 amino acids in length.

6. A method according to claim 5 wherein the first binding substance comprises an extracellular binding domain of the natriuretic receptor GC-A (SEQ ID NO: 34).

7. A method according to any one of claims 2 to 4 wherein the first binding substance comprises an antibody or a fragment or derivative thereof.

8. A method according to claim 7 wherein the antibody comprises a polyclonal antibody, monoclonal antibody, oligoclonal antibody, bifunctional antibody or crossreacting polyclonal antibody.

9. A method according to any one of claims 3 to 8 wherein in the agent, (a)(i) is SEQ ID NO. 3 and (b)(i) is SEQ ID NO: 6 or (a)(i) is SEQ ID NO. 2 and (b)(i) is SEQ ID NO. 5.

10. A method according to any one of claims 3 to 8 wherein the agent comprises or consists of:

(a) $proBNP_{15-24}$ and $proANP_{82-96}$;

(b) $proBNP_{1-37}$ and $proANP_{29-98}$;

(c) $proBNP_{10-29}$ and $proANP_{20-80}$;

(d) $proBNP_{1-76}$ and $proANP_{1-98}$;

(e) $proBNP_{10-29}$ and $proANP_{60-80}$;

(f) $proBNP_{1-108}$ and $proANP_{1-126}$; or

(g) $proBNP_{77-92}$ and $proANP_{112-126}$.

11. A method according to any one of claims 3 to 10 wherein the agent is a polypeptide.

12. A method according to any one of claims 2 to 11 wherein the first binding substance and/or the agent is:

(a) labelled with a detectable label; and/or

(b) immobilised.

13. A method according to any one of claims 2 to 12 which additionally comprises contacting the sample with a second binding substance which is able to bind to the first binding substance.

14. A method according to claim 13 wherein the second binding substance is:

(a) labelled with a detectable label; and/or

(b) immobilised.

15. A method according to claim 13 wherein the second binding substance causes precipitation of the first binding substance and any peptide which is bound to it.

16. A method according to any one of the preceding claims which comprises an immunoassay.

17. A method according to any one of the preceding claims thereby to diagnose heart failure or monitor treatment of a cardiac condition.

18. An agent which comprises:

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length; and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5), NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.

19. An agent according to claim 18 which comprises or consists of:

(a) $proBNP_{15-24}$ and $proANP_{82-96}$;

(b) $proBNP_{1-37}$ and $proANP_{29-98}$;

(c) $proBNP_{10-29}$ and $proANP_{20-80}$;

(d) $proBNP_{1-76}$ and $proANP_{1-98}$;

(e) $proBNP_{10-29}$ and $proANP_{60-80}$;

(f) $proBNP_{1-108}$ and $proANP_{1-126}$; or

(g) $proBNP_{77-92}$ and $proANP_{112-126}$.

20. An agent according to claim 19 which comprises any one of SEQ ID NOs. 13, 14, 15, 17, 18, 19 or 20.

21. An agent according to any one of claims 18 to 20 which is labelled with a detectable label.

22. A polypeptide agent according to any one of claims 18 to 21.

23. A polynucleotide comprising sequence which encodes a polypeptide according to claim 22 or sequence which is complementary to the coding sequence.

24. A polynucleotide according to claim 23 which comprises:

(a) (i) SEQ ID NOs. 7, 8 or 9;

(ii) a sequence complementary to (i);

(iii) a sequence which hybridises under stringent conditions to (i) or (ii);

(iv) a sequence which is degenerate as a result of the genetic code to (i), (ii) or (iii);

(v) a sequence having at least 70% identity to any of the sequences in (i) to (iv); or

(v) a fragment of any of the sequences in (i) to (v);

and

(b) (i) SEQ ID NOs. 10, 11 or 12;

(ii) a sequence complementary to (i);

(iii) a sequence which hybridises under stringent conditions to (i) or (ii);

(iv) a sequence which is degenerate as a result of the genetic code to (i), (ii) or (iii);

(v) a sequence having at least 70% identity to any of the sequences in (i) to (iv); or

(vi) a fragment of any of the sequences in (i) to (v).

25. An expression vector comprising a polynucleotide according to claim 23 or 24.

26. A host cell comprising a polynucleotide according to claim 23 or 24 or an expression vector according to claim 25.

27. A process for producing a polypeptide according to claim 22 which process comprises:

(a) cultivating a host cell according to claim 26 under conditions to provide for expression of the polypeptide; and optionally

(b) recovering the expressed polypeptide.

28. A process for producing a polypeptide according to claim 22 which comprises chemical synthesis.

29. A method of identifying a substance that binds specifically to

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5), NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70.% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length which method comprises:

(A) contacting a candidate substance with (a) and (b) under conditions which allow specific binding; and

(B) determining whether the candidate substance binds to (a) and (b).

30. A method according to claim 29 which comprises:

(a) contacting the candidate substance with an agent according to any one of claims 17 to 21; and

(b) determining whether the candidate substance binds to the agent.

31. A bi- or oligo- specific antibody, fragment or derivative thereof which is able to bind to both:

(a) (i) proANP (SEQ ID NO. 1), ANP (SEQ ID NO. 2) or NT-proANP (SEQ ID NO. 3);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length; and

(b) (i) pro-BNP (SEQ ID NO. 4), BNP (SEQ ID NO. 5) or NT-proBNP (SEQ ID NO. 6);

(ii) a homologous sequence having at least 70% identity to (i); or

(iii) a fragment of (i) or (ii) which is at least 6 amino acids in length.

32. An antibody, fragment or derivative according to claim 31 which is labelled with a detectable label.

33. A process for making an antibody as defined in claim 31 or 32 comprising culturing a cell that expresses the antibody and optionally purifying antibody from the cell.

34. A process according to claim 33 in which the cell is one which is obtainable by administering a polypeptide according to claim 22 to a mammal, extracting B cells from the mammal and selecting a cell from these based on the ability to express an antibody with the specificity of the antibody of claim 31.

35. A process according to claim 33 in which the cell is recombinant for a polynucleotide which expresses the antibody.

36. A solid support comprising an antibody according to claim 31 or 32.

37. A solid support according to claim 36 which is a particle, dipstick or microtitre plate.

38. An agent according to any one of claims 17 to 22, a polynucleotide according to claim 23 or 24 or an antibody, fragment or derivative thereof according to claim 31 or 32 for use in treatment of the human or animal body by diagnosis or monitoring of treatment.

39. Use of a first binding substance as defined in any one of claims 2 to 8, an agent according to any one of claims 18 to 22, a polynucleotide according to claim 23 or 24 or an antibody according to claim 31 to 32 for the manufacture of a reagent for diagnosis and/or monitoring treatment of heart failure.

40. A diagnostic kit comprising:

(a) a first binding substance as defined in claim 2; or

(b) a first binding substance and an agent as defined in claim 3;

wherein optionally the binding substance and/or the agent is labelled.

41. A kit according to claim 40 wherein the first binding substance is as defined in any one of claims 4 to 8, and/or is present on a solid support according to claim 36 or 37.

42. A kit according to claim 40 or 41 wherein the agent is as defined in claims 18 to 22.

43. Use of a first binding substance as defined in any one of claims 2 to 8, an agent according to any one of claims 18 to 22, a polynucleotide according to claim 23 or 24, an antibody, fragment or derivative according to claim 31 or 32, a solid support according to claim 36 or 37 or a kit according to any one of claims 40 to 42 in a method for diagnosis and/or monitoring treatment of heart failure.

44. A method of diagnosing and/or monitoring treatment of heart failure in an individual comprising:

(a) obtaining a biological sample from an individual;

(b) determining the activation or inactivation of both the ANP and BNP hormonal systems in the individual by a method which comprises simultaneously detecting the presence or amount of proANP and proBNP or fragments thereof in the sample.

45. Use according to claim 43 or a method according to claim 44 which comprises a method as defined in any one of claims 1 to 17.